FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
MC-158.USA	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	
09/914669	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/05454	03 March 2000	03 March 1999

TITLE OF INVENTION

HERPES SIMPLEX VIRUS REACTIVATION MODEL

APPLICANT(S) FOR DO/EO/US

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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☒ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Associate Power of Attorney
Unsigned Declaration and Power of Attorney

PCT/PTO 31 AUG 2001

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/914669	PCT/US00/05454	MC-158.USA

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1000.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$860.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$710.00	
☒ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$690.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$690.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	17 - 20 =	0	x $18.00	$0.00	
Independent claims	5 - 3 =	2	x $80.00	$160.00	
Multiple Dependent Claims **(check if applicable).**			☒	$270.00	
TOTAL OF ABOVE CALCULATIONS =				$1,120.00	
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$560.00	
SUBTOTAL =				$560.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				$0.00	
TOTAL NATIONAL FEE =				$560.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				$0.00	
TOTAL FEES ENCLOSED =				$560.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$560.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-4213** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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43,924

REGISTRATION NUMBER

August 30, 2001

DATE

3/prts

HERPES SIMPLEX VIRUS REACTIVATION MODEL

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of the filing of U.S. Provisional Patent Application Serial No. 60/122,961, entitled *UV-B Light Induces Reactivation In a Murine Model of Cutaneous HSV-1 Infection*, filed on March 3, 1999, and the specification thereof is incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention (Technical Field):

The present invention relates to methods and systems for animal models of reactivation of herpes simplex virus infections, including models for evaluation of the effectiveness of drugs to inhibit reactivation infections or limit the severity or duration of reactivation infections. The present invention also relates to methods and systems for animal models to test the effectiveness of sunscreens and other substances providing protection against ultraviolet radiation.

Background Art:

Herpes simplex virus (HSV-1 and HSV-2) infections are common chronic infections of humans, yet are difficult to study in the human host. HSV infections are initiated by cutaneous or mucous membrane exposure to live virus. After a 2 to 8 day incubation period, the primary infection is manifest by characteristic vesicular lesions on an erythematous base. The lesions progress to form sharply demarcated ulcers and crusts. After the primary lesions heal, there is lifelong latency, with persistence of the genome within trigeminal and sacral nervous system ganglia. The latent virus can reactivate in response to environmental or physiologic stimuli including fever, mechanical trauma and ultraviolet radiation (UVR). Reactivated disease ranges from subclinical epithelial shedding of low titer virus to readily evident mucocutaneous vesicular lesions.

The events that induce HSV reactivation are difficult to study in humans because of 1) the difficulty in identifying patients with very early disease, 2) the kinetics of reactivation events are unpredictable, 3) not all individuals will reactivate in response to the same stimuli and 4) the exact location of lesion formation is not observable until vesiculation occurs, which is a late event in reactivated disease. Thus, animal models are critical to provide insight into the disease process. Ganglia explant experiments have provided information regarding the series of viral and cellular events leading to virus expression but in vivo models have been more difficult to develop.

The mechanism of UV induced reactivation is unknown. UV radiation is known to impair both local and systemic immune responses. Initial studies investigated the association between UV-light induced carcinogenesis and immunosuppressive effects. Later studies demonstrated that UV

radiation to skin results in protean effects on immunity including depression of adhesion molecules vital for cell emigration, and release of immunosuppressive cytokines. In both murine and human keratinocytes, multiple cytokines may be produced in response to UV irradiation including IL-1α, IL-6, IL-8, IL-10, IL2, TNF-α, TGFβ, NGF, and basic fibroblast growth factor. The majority of cytokine activity remains local, with the exception of IL-1 and IL-10, and to some degree IL-6, which may have a measurable systemic effect. In HSV infection models, mice pretreated with UV-B irradiation at the site of subsequent HSV cutaneous inoculation exhibited decreased proliferative T cell responses and increased severity of disease compared to non-UV exposed controls. Yasumoto S et al: *J Immunol* 139(8):2788-93 (1987); Aurelian L et al: *J Virol* 62(7):2520-4 (1998); El-Ghorr A A and M Norval: *J Gen Virol* 77(Pt. 3):485-91 (1996).

A murine model of UV induced HSV-1 skin reactivation was first described in 1987. Mice immunosuppressed by pretreatment with UV for three days prior to infection were subjected to a cutaneous inoculation. Following healing, the infected mice were then exposed to multiple irritants including shaving, further UV irradiation and tape-stripping of the original inoculation site. Approximately 80% of the mice developed lesions consistent with HSV reactivation, with lesions confined to the site of initial inoculation and the immediately adjacent area. Norval M et al: *J Gen Virol* 68(Pt. 10):2693-8 (1987). A guinea pig model of HSV-2 vaginal infection developed recurrent herpes genitalis in 60% of exposed animals following genital UV irradiation. Stanberry L R et al: *J Infect Dis* 46:397-404 (1982). Other infection models include both rabbit and mouse keratitis. Herpetic keratitis may be stimulated to reactivate by UV in up to 80% of mice following corneal inoculation with HSV-1.

Humans subjected to UV B irradiation of lip or peri-genital regions have been stimulated to reactivate HSV-1 or -2 respectively. Rooney J F et al: *J Infect Dis* 166(3):500-506 (1992); Spruance S L: *J Clin Micro* 22:366-68 (1985). Human studies of HSV reactivation were first reported in 1975, when three patients with a history of recurrent HSV on the buttock or thigh developed reactivation after high dose UV directed to their usual reactivation site. In further studies characterizing UV induced eruptions in humans, it was determined that 3 minimal erythemal dose (MED) of UV light directed to the site of previous reactivation would induce lesions approximately 60% of the time, with lesions appearing 3-6 days post UV for HSV-2 and 1-6 days post UV for HSV-1. Lesions appeared within the UV exposed area, to 5–10 mm outside the UV exposed area but within local innervation sites. Virus was recovered from early vesicles approximately 75% of the time.

Because the mechanism of UV induced cutaneous reactivation is not fully understood and results in significant morbidity to humans, there is a need for a reliable cutaneous reactivation model. A model, such as an animal model system, that induces HSV reactivation disease after

physiologically relevant stimuli such as UVR would allow investigation into the mechanisms by which environmental stimuli to the skin resulting in signals to the sensory ganglia, and subsequent virus reactivation. Such a model could also be used to evaluate the efficacy, in a biological system, of sunscreens and other UVR protectants.

Accordingly, it is a primary object of the present invention is to provide an animal model of reactivation of HSV infection upon exposure to determined stimuli.

A further object of the present invention is to provide an animal model of reactivation of HSV infection upon exposure to determined stimuli whereby the efficacy, dosing, route of administration or frequency of administration of drugs and substances intended to inhibit reactivation infections or limit the severity or duration of reactivation infections may be evaluated.

A further object of the present invention is to provide a cutaneous animal model of reactivation of HSV infection upon exposure to UVR.

A further object of the present invention is to provide an animal model for determination of the effectiveness of sunscreens and other substances providing protection against UVR, whereby inhibition of reactivation of HSV infection upon exposure to UVR is a measure of the effectiveness of the sunscreen or other substance.

A primary advantage of the present invention is that it provides a method and system in a cutaneous animal model for reactivation of HSV infection, modeling the course of the disease in humans.

Another advantage of the present invention is that it permits the rapid and efficient screening of drugs to inhibit HSV reactivation and to reduce the severity or duration of reactivation infections.

Another advantage of the present invention is that it provides a method and system for discovery of drugs that inhibit HSV reactivation.

Another advantage of the present invention is that it provides a biological system with defined endpoints for determining the efficacy of sunscreens and other substances in protecting against exposure to determined amounts of UVR.

Another advantage of the present invention is that it provides a method and system in an animal model for study viral diseases in the nervous system and nervous system diseases and

syndromes, including determining the efficacy of drugs and treatment regimens for nervous system diseases and syndromes.

Other objects, advantages and novel features, and further scope of applicability of the present invention will be set forth in part in the detailed description to follow, taken in conjunction with the accompanying drawings, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention. The objects and advantages of the invention may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating a preferred embodiment of the invention and are not to be construed as limiting the invention. In the drawings:

Fig. 1 is a table showing reactivation rates of HSV infected SKH-1 mice;

Fig. 2 depicts HSV DNA, amplified by PCR, from infected mouse skin samples; and

Fig. 3 is a table showing recovery of live virus at specified days post-reactiviation exposure to UVR.

DESCRIPTION OF THE PREFERRED EMBODIMENTS (BEST MODES FOR CARRYING OUT THE INVENTION)

The invention provides an animal model of HSV reactivation disease that is analogous to human HSV-1 cutaneous reactivation disease. In one embodiment, a murine model of HSV reactivation disease in hairless mice is utilized. The mice may be SKH-1 mice, which are immunocompetent and manifest appropriate inflammatory responses, including cytokine responses and delayed-type hypersensitivity to various challenges. Other rodent strains may be employed, including but not limited to other strains of mice. Transgenic mouse strains, including strains incorporating human genetic material, may also be employed.

In one embodiment, HSV-1 strain 17 syn+ was applied to a superficial dermabrasion on the lateral abdominal wall of SKH-1 mice. Inoculated mice developed a primary infection resulting in a "zosteriform" lesion pattern, which resolved in the majority of mice. HSV reactivation was initiated by exposing mice that recovered from the primary infection to 2 MED of UVR at the site of primary

infection. Four days post UV irradiation, lesions erupted in a widespread pattern along the flank. Lesions persisted for 3 to 5 days and then healed completely, and could be reactivated with subsequent re-exposure to UVR. The frequency of reactivation was approximately 60%. Mortality rate was 30% for the primary infection and 4% for reactivated disease. Photoreactivated disease was demonstrated in Balb/C and B6129 mouse strains, with reactivation rates approximating 60%. In all mouse strains, histology of reactivated lesions demonstrated localized areas of vesicles, ulcers and inflammation. HSV was detected in early-reactivated lesions by PCR and virus culture, while no HSV was detected prior to UV exposure. Uninfected control mice exposed to UV did not develop gross or microscopic lesions.

The histologic changes in UV reactivated disease in this mouse model are similar to lesions seen in late primary disease. Specifically, foci of ulceration and dermal inflammation are observed in reactivated disease. These changes did not occur in uninfected UV exposed mice. Reactivated disease was generally milder and mortality was significantly lower than for primary disease (4% versus 30%, respectively). This is similar to human HSV infection, in which reactivation disease is less severe than primary disease.

HSV antigen in late reactivated lesions is below levels readily detectable from background by standard immunohistochemistry. Consistent with this was that HSV cultures from reactivated lesions yielded low levels of virus only, 40 to 60 pfu per sample. By both culture and PCR, HSV was detectable only from early reactivated lesions and was no longer demonstrable by the time the inflammatory reaction and epithelial necrosis was seen. Similar findings have been described in human reactivated disease, where diagnosing reactivated disease may be problematic as virus quantity is significantly lower than in primary disease and virus is present only in early macular and vesicular lesions.

Mice exposed to UV on the non-infected side failed to reactivate clinically apparent lesions. The mice subsequently reactivated at the expected frequency and with the expected pattern of lesions upon UV exposure of the infected side, indicating that the primary infection and establishment of latency was normal. Together, these findings suggest that UV exposure was only effective at inducing reactivation when applied to the area of previous primary disease and that systemic immunosuppressive effects of UV irradiation were not responsible for HSV reactivation from dorsal root ganglia. The immune events resulting in reactivation from latency are thus likely due to UV-induced changes in the local cutaneous milieu and subsequent signaling to the neurons.

Applications of this murine cutaneous reactivation model include studies characterizing local immune events in skin and spinal ganglia occurring during HSV reactivation, and trials of preventive and therapeutic agents designed to limit reactivation disease.

The method of this invention may be employed to determine the effectiveness of a composition to inhibit herpes simplex virus infection reactivation. One or more animals are obtained, and a superficial dermabrasion is created on the animal. Any effective means to create a superficial dermabrasion may be employed, including use of cutting or wire brush implements. In one preferred embodiment, a high-speed rotary wire brush is employed to create an approximately 1 cm long superficial linear dermabrasion. The animal is then inoculated with HSV by application of a composition including herpes simplex virus to the superficial dermabrasion, which thereby results in a primary herpes simplex virus infection in the animal. In this and other methods of this invention, either HSV-1 or HSV-2 may be employed, and where desired specific strains of HSV may be employed, including strains with which a specific patient is infected, and it is desired to determined the susceptibility of such strain to various treatment options. In this and other methods of this invention, the quantity of HSV applied to the superficial dermabrasion results in death of 50% or fewer of animals due to the primary infection, and in a preferred embodiment the quantity of HSV applied is at least one-half log less than the quantity of HSV which results in death of 50% of the animals due to primary infection. Following application of the HSV and primary infection, the superficial dermabrasion is permitted to heal and the primary HSV infection allowed to resolve. The HSV infection can be reactivated by exposing the area of superficial dermabrasion to UVR. In this and similar methods, UV-B or solar spectrum UV may be employed. In one preferred embodiment, solar spectrum UV is employed at 2 MED. Either prior to exposure to UVR or subsequent to exposure to UVR, the composition to be tested for inhibition of HSV infection reactivation is administered to the animal. If administered after exposure to UVR, the composition to be tested may preferably be administered prior to clinical evidence of reactivation. It is then determined whether the HSV infection has been reactivated, and by comparison to control groups, the rate of inhibition of HSV reactivation is determined. The severity and duration of HSV reactivation infection may similarly be determined, and compared to control groups or groups receiving other treatments.

The foregoing method may be employed with any composition desired to be tested for inhibition of HSV reactivation infection, or for limiting the severity or duration of the HSV reactivation infection. The composition may thus include an HSV vaccine, an anti-viral agent, an immune system stimulatory agent, and the like.

In a related method, the effectiveness of a composition to inhibit primary HSV infection may similarly be determined. In this method, a composition to inhibit primary HSV infection is administered, either prior to inoculating the animal with HSV, or subsequent to such inoculation but prior to expression of clinical disease. It is then determined whether the rate of primary disease has been inhibited, and similarly whether the severity or duration of primary infection has been decreased, all in reference to control animals. It is also possible and contemplated that the

effectiveness of a composition to inhibit primary HSV infection may be further determined by attempted reactivation by UVR, and determining whether the HSV infection is reactivated, and if so, by determining the severity or duration of the reactivation infection.

In yet another embodiment, the methods of this invention may be employed to determine an effective dose of a composition to inhibit herpes simplex virus reactivation. Two or more animals are obtained, and a superficial dermabrasion is created followed by HSV inoculation, resulting in a primary HSV infection. The animals are then treated, with the schedule of administration, route of administration and quantity of drug varied as desired, to determine the optimal effective dose. Treatment may commence before exposure to UVR to reactivate the HSV infection or subsequent to UVR exposure, or where multiple doses or time release drugs are employed, treatment may be both before and after exposure to UVR. Any schedule of administration and route of administration known in the art may be employed, including, without limitation, oral, dermal, mucosal, topical, i.v., s.c., i.m., peritoneal and related routes of administration. The rate of rate of reactiviation of HSV infection for each selected dose is then determined, so that an optimal dose, route of administration or quantity of drug may be determined.

In yet another embodiment, the effectiveness of a UV protectant may be determined using the methods of this invention. One or more animals are obtained, and a superficial dermabrasion created and primary HSV infection induced as described above. After the superficial dermabrasion has healed and the primary HSV infection resolved, an UV protectant is then administered to the animal. The area of superficial dermabrasion is then exposed to UV radiation, and it is subsequently determined whether the HSV infection is reactivated. The UV protectant may be either a topical agent, including a sunscreen agent contain UV-B, UV-A or other UV spectrum screens, or may be a systemic or other agent.

The methods of the invention may also be employed to determine the effectiveness of a composition to provide central nervous system protection. In this method, a composition to provide central nervous system protection is administered, either prior to inoculating the animal with HSV, or subsequent to such inoculation but prior to expression of clinical disease. It is then determined whether the rate of central nervous system damage has been inhibited, and similarly whether the severity or duration of such damage has been decreased, all in reference to control animals. For this application, in general higher quantities of HSV must be utilized for the primary infection in order to induce a statistically desirable and significant rate of central nervous system injury resulting from HSV in control or untreated animals.

Industrial Applicability:

The invention is further illustrated by the following non-limiting examples.

Example 1 Animal Strains Employed

Three strains of mice were tested: SKH-1, Balb/C, and B6129. SKH-1 hairless mice (Charles Rivers) are an outbred, albino hairless mouse strain originally developed by Temple University and now maintained by Charles Rivers Laboratory. SKH-1 may be preferentially used due to its long history in photobiology experimentation and because the hairless phenotype results in ready evaluation of skin lesions. Balb/C mice (Jackson Labs) are an inbred, albino haired mouse strain and B6129 (Taconic, Germantown, NY) are a pigmented, haired F1 hybrid from C57Bl/6 x 129S6/SvEv parents. B6129 may be employed in studies involving non-inbred knockout models, including DNA repair deficient models.

Example 2 Induction of Primary Infection

Mice were anesthetized by intraperitoneal injections of 80 mg/kg of ketamine and 16 mg/kg xylazine (ICN Biomedicals Inc.) or Avertin, 250 mg/kg (Aldrich) prior to all invasive procedures. A 1 cm long superficial linear dermabrasion to the lateral abdominal wall was created using a small wire brush attached to a cordless rotary tool set at 5,000 rpms (Dremel Minimite Model 750) on 6 to 8 week old mice, weighing 20 to 30 grams. A 50 microliter inoculum of HSV-1 17 syn + at various concentrations was applied to the abraded area via pipette, gently rubbed into the dermis with a minimal essential medium (MEM) soaked dacron-tipped swab and allowed to dry. In haired mouse strains (Balb/C and B6129), the lateral abdominal wall was shaved prior to dermabrasion.

Mice were observed daily for signs of HSV-1 infection. Erythema, vesicles and ulcerations were considered clinical evidence of disease. Changes to the epithelium were recorded as mild, moderate or severe depending on extent of lesions. Mild disease (1+) was defined as non-coalescing discrete lesions in a zosteriform pattern. Moderate disease (2+) was defined as discrete lesions that form ulcers that do not completely coalesce. Severe disease (3+) included coalescing, full thickness hemi-circumferential. Mice with severe skin lesions and evidence of myelitis (4+) such as gut stasis or hind limb paralysis were euthanized. The ID 50 (quantity of virus in pfu resulting in 50% mortality) was established for each strain tested:

Mouse strain	Incubation period	Time to healing	ID 50
SKH-1	3-10 days	4 to 8 days	120 pfu
B6129	3 –8 days	4 to 8 days	700 pfu
Balb/C	3-7 days	4 to 8 days	600 pfu

Lesions appeared 4 to 6 days post inoculation. Initial lesions were erythematous macules that progressed to small vesicles on an erythematous base. Vesicles rapidly developed into sharply demarcated ulcers over a 1 to 2 day period and spread from the abdominal wall laterally in a zosteriform manner to the spinal ridge, but did not cross the midline. In mice surviving the initial

infection, skin healing was complete with little to no visible scarring. An inoculation dose of 60 pfu reliably infected 95% of SKH-1 mice (n= 80), with a mortality rate of 30%. Death was due to necrotizing myelitis resulting in gut stasis or hindlimb paralysis. In survivors, disease was rated as mild in 10 mice, moderate in 27 mice, and severe in 19 mice.

Example 3 Histology of primary disease

Formalin-fixed skin was embedded in paraffin, cut into 4.6 μm sections then stained with hematoxylin and eosin. Microscopic examination of the earliest apparent lesions showed foci of pale staining, enlarged and disorganized keratinocytes. Within these foci, epithelial syncytia were observed. Eosinophilic intranuclear inclusion bodies in degenerating cells were occasionally present. At this stage, modest acute inflammation in the affected epithelium and underlying dermis and small numbers of dyskeratotic epithelial cells were present. As the epithelium degenerated, lesions progressed to vesicles and ultimately coagulative necrosis. The epithelium sloughed, forming ulcers covered with crusts of necrotic debris. The underlying dermis became heavily infiltrated with mixed inflammatory cells. Lesions remained localized and were sharply demarcated from surrounding normal skin. Histologically, mouse HSV primary disease was similar to disease described for cutaneous HSV infection in humans.

Example 4 Histology, spinal cord

The spinal cord of a mouse showing flaccid paralysis of the hindlimbs was examined. In the thoracic cord, an extensive area of malacia was present, extending from the level of the central canal dorsal to the border of the spinal cord and involving large areas of the dorsal column of white matter. Dorsal column involvement was bilateral, although one side was more severely affected than the other. In areas of malacia, a variable degree of myelin loss occurred: some areas were completely devoid of myelin, other areas had focal areas of severe demyelination. A proliferation of glial cells, neuronal necrosis, and neuronophagia occurred, and inflammatory cells and glitter cells were rare. Spinal nerves adjacent to the affected area showed marked gliosis and a few lymphoid cells were present. In the cauda equina, a few dilated and empty axon sheaths were apparent. The necrotizing myelitis was consistent with an HSV etiology.

Example 5 Immunohistochemistry, primary disease

For immunohistochemistry, slides were heat fixed for one hour at 55° C then decerated and stained for HSV using a rabbit primary antibody (Dako #B 0114, rabbit anti-HSV 1) and a goat anti-rabbit secondary antibody (Vector Vectastatin Elite ABC kit). HSV-1 specific immunohistochemistry staining (IH) localized herpes antigen to epidermal lesions. Surface epithelium and hair follicles in vesicles and foci of epidermal necrosis were strongly immunoreactive with antibodies against HSV-1. Immunoreactivity did not extend into surrounding normal skin. Both cytoplasm and nuclei of cells

were immunopositive, with nuclear staining often more prominent than cytoplasmic. With maturation of lesions to ulceration and sloughing of surface epithelium, immunoreactivity disappeared except in hair follicles. Occasional cutaneous nerve fibers in proximity to the initial lesions exhibited immunoreactivity. Immunohistochemistry staining of the spinal cord failed to reveal HSV immunoreactivity in neurons and nerve fibers, even when using high concentrations of the primary antibody. Rare phagocytic microglia in areas of malacia gave positive antibody staining.

Example 6 DNA extraction

Genomic DNA was extracted from skin samples snap frozen in liquid nitrogen immediately after harvest. Skin samples (~20 mg each) were disrupted with a liquid nitrogen-cooled mortar (Bel-art products cat# F37260) and pestle and processed using a QIAGEN DNA mini column kit (Quigen Inc, Santa Clara CA) to yield approximately 40 μg DNA per 20 mg skin specimen. DNA was quantified by optical density measurements at OD 260 nm and assessed by agarose gel electrophoresis and ethidium bromide staining.

Recombinant expressed proteins encoded by the carboxy terminus regions of glycoprotein B (gB-SS1, aa 228-903), glycoprotein D (gD-PB1, aa 306-462) and a 14,300 - 200,000 kDa prestained protein molecular weight marker series (Gibco BRL) were separated on an SDS -12.5% polyacrylamide gel in a mini-PROTEAN II electrophoresis cell (Bio-Rad) and transferred onto a 0.2 μm nitrocellulose membrane by standard techniques. All recombinant expressed proteins were generated using pATH expression vectors. Blood and serum samples were diluted 1:200 in a buffered milk solution and incubated with blots overnight at 4°C, and developed by standard techniques using a goat anti-mouse alkaline phosphatase conjugated secondary antibody (Southern Biotechnology).

For qualitative determination of HSV by PCR, genomic DNA was amplified using PCR primers designed to amplify a region of the HSV-1 pol gene from nt 1930 to 2191. The sense strand sequence was 5' - CAT GAC CCT TGT GAA GTA CG - 3' (SEQ ID NO: 1), antisense was 5' - GCT CGA GAG CTT GAT CTT GTC G – 3' (SEQ ID NO: 2). Running conditions were 95°C for 60 seconds, then 95°C, 64°C and 72°C for 45 seconds each for 30 cycles, followed by a 10 minute extension at 72°C. (Perkin Elmer, model 480).

Example 7 Serology

Serum antibody responses to infection were demonstrated by Western blot. By day 7 post inoculation, IgM antibody responses to a peptide encoded by the carboxy terminus region of glycoprotein B (gB SS1) were detectable in infected mice. IgG antibodies to regions of glycoprotein B and glycoprotein D were demonstrated in all infected mice with clinically evident lesions by 21 days

post inoculation (n = 52). Four mice had either very mild or no clinically evident disease but developed antibodies specific to gB and gD. Of these 4 mice, 2 reactivated clinically apparent lesions on UV challenge. Control mice (n = 12) underwent the dermabrasion procedure but had only the vehicle without virus applied to their skin lesions. These mice did not seroconvert and no IgM or IgG herpes specific antibodies were detected on serial studies.

Example 8 Culture, primary disease

Live virus was recovered from swabs of skin or from skin or tissue biopsy material. Specimens were plated on standard Vero cell monolayers and observed for virus cytopathic effect. All cultures demonstrating cytopathic effect were confirmed by IH. HSV-1 was recovered from early macular and vesicular skin lesions and ulcers but not from later crusts or healing lesions. Control mice housed in separate cages did not develop lesions, and remained culture negative.

Example 9 Induction of Reactivation

The primary infection progressed through complete healing prior to reactivation stimuli. Mice undergoing reactivation were first anesthetized and the face and eyes were shielded. Haired mouse strains were shaved along the flank and lateral abdominal wall. Two light sources were utilized. Mice exposed to UV-B were placed with the infected side down on an ultrabrite transilluminator (Ultra-Lum, model UVB-15) covered by a UV transparent glass protector (Uvi Clear). Exposure was for 30 seconds of UV-B radiation (2100 J/m 2, peak wavelength at 312 nm) as determined by a spectrophotometer, a dose equivalent to 2 MED. Alternatively, mice were placed prone and the entire back exposed to 2 MED of UVR from a UV-340 light source, which emits a UV spectrum nearly identical to that of a solar simulator and emits a very limited non-solar UV spectrum. Control mice underwent the dermabrasion procedure but did not have live virus applied to the area of dermabrasion. Controls were exposed to identical UV doses.

Example 10 Rates of Reactivation - Survivors of ID 50 Experiment

SKH-1 mice (n = 9) that were survivors of an ID 50 dosing experiment that initially received an inoculation of between 3 to 3,000 pfu were employed. These mice were irradiated 22 to 30 weeks after the primary infection and were 30 to 42 weeks of age; no spontaneous reactivation was noted prior to UV-B irradiation. Reactivation after UV exposure occurred in 7 of 9 mice; 2 exhibited severe disease and required euthanasia. Reactivated lesions consisted of erythematous macules, small vesicles, ulcers and crusts. After healing of the UV induced lesions, 3 out of 6 mice reactivated on subsequent UV re-challenge. Disease in one mouse was successfully reactivated following four sequential UV exposures. HSV uninfected mice of identical age (n = 5), that had undergone the dermabrasion procedure but did not receive virus, were exposed to the same UV-B dose. Control mice did not exhibit erythema, induration, vesicles or ulcers. Minimal skin flaking was noted on the UV exposed side approximately day 3 post irradiation.

Example 11 Rates of Reactivation - HSV infected SKH-1 mice to quantify reactivation frequency

Twenty mice aged 11 to 13 weeks were infected with 60 pfu HSV-1. Fifteen mice survived the primary infection and were exposed to 2 MED of UV light with a peak wavelength of 312 nm. Five of fifteen (33%) reactivated. Dermabraded but uninfected control mice (n = 4) who received an identical UV dose remained lesion free.

Example 12 Evaluation of UV Induced Systemic Immunosuppression

The potential role of UV induced systemic immunosuppression on local HSV reactivation was evaluated. SKH-1 mice (n = 24) were infected with 60 pfu. Sixteen mice survived the primary infection and were allowed to heal for two weeks, then exposed to 2 MED of UV light directed to the uninfected side away from the site of dermabrasion. Twice daily examination for two weeks did not reveal any evidence of clinically reactivated lesions. Subsequent UV B exposure to the infected side resulted in clinically apparent reactivation four days post-UV, with erythematous macules, small vesicles and shallow ulcers in 9 of the 16 mice. The data is included in Fig. 1. Control mice (n = 4) remained lesion free.

Example 13 Rates of Reactivation - Mice infected at one half log below the ID 50 and exposed to solar spectrum light

HSV-infected SKH-1 mice was used to evaluate reactivation frequency using solar spectrum UV. This group of mice (n = 20) ranged in age from 3 to 12 months, and were 2 to 10 months post primary infection. All had been previously subjected to UV exposure using the UVB light source of Example 9. No mice had prior evidence of spontaneous clinical reactivation. The mice were exposed to 2 MED from a solar spectrum source and evaluated for reactivation. Twelve mice (60%) developed lesions consistent with HSV reactivation by day 4 post UV. The data is included in Fig. 1. Four control mice developed mild erythema that faded after 24 hours and mild skin flaking.

Example 14 Reactivation Frequency

The overall reactivation frequency for the three groups of SKH-1 mice (survivors of a ID-50 experiment, mice exposed to UV B at 312 nm and mice exposed to solar spectrum UV) was 55%. Reactivation rates for each group were 78%, 45% and 60%, respectively. The highest reactivation rates were seen in a small group of ID 50 experiment survivors, who had a prolonged delay between initial infection and first UV B exposure compared to other groups (30-42 weeks versus 11-13.5 weeks, respectively) (see Fig. 1). Comparing the reactivation rates in UV exposed mice between groups 1 and 2, there was a significant difference in reactivation, which may be due to advanced age at time of UV stimulus (p=0.04, Fischer's exact test). The rates of reactivation did not differ statistically between other groups.

Example 15 Reactivated disease, Balb/C and B6129

To test reactivation in haired models, Balb/C and B6129 mice underwent a primary infection as previously described in Example 2. Mice were allowed to fully heal. To control for HSV reactivation due to skin irritation, infected mice were randomized to either shaved and UV exposed versus shaved and unexposed groups. Clinically apparent reactivation was evident by day 4 post UV exposure. In B6129 mice, a total of 9 mice were shaved and exposed to 2 MED of UV light from a solar spectrum lamp; 4 of 9 developed characteristic lesions positive for HSV-1 by culture. Four HSV infected mice underwent shaving but did not receive UV exposure. No reactivation events occurred in these mice.

In all mouse strains tested, photoreactivated disease consisted of lesions which appeared in a widespread distribution on the flank by approximately day 4 post UV. The lesions began as macules that progressed to small vesicles on an erythematous base, consistent with HSV disease. Vesicles resolved or progressed to ulcers and crusting. The overall duration of disease was approximately 4 days. The reactivated disease was generally milder and shorter in duration than primary disease. The mortality rate from reactivated disease was less than 5%. UV irradiated control mice displayed no lesions, and had only a very mild, fine flaking of the skin post-UV.

Example 16 Histology, Reactivation

Reactivated lesions showed foci of discrete vesicles. Later lesions showed prominent focal ulcerations with overlying crusts of necrotic debris. The dermis beneath areas of ulceration contained modest numbers of mixed inflammatory cells. Skin sections from sham infected control mice exhibited only hyperkeratosis after exposure to UV light. No foci of inflammation, ulceration or epithelial degeneration were identified in either control mice or in skin from infected mice that was collected from the uninfected side.

Example 17 Determination of HSV in reactivation lesions

HSV was confirmed in reactivation lesions by culture and PCR. Virus was recovered from skin swabs and biopsies beginning day 3 post UV, corresponding with the onset of earliest visible skin lesions. By onset of crusting and lesion healing, approximately day 7 post UV, virus was no longer readily detectable. Culturable virus was obtained from 67% of early reactivated lesions (14 of 20 specimens). The results day-by-day post-UV exposure are shown in Fig. 3. No virus was detected day 1 or 2 post UV. Standard Vero cell cultures required up to 5 days incubation prior to onset of cytopathic effect, and an average of 50 pfu of virus was recovered per 8 mm skin lesion biopsy. In contrast, cultures of primary lesions demonstrated high levels of virus and CPE was evident by 24 to 48 hours after inoculation. UV exposed control mice housed in separate cages did not develop lesions and remained culture negative. The time course and cytopathic effect seen in skin cultures from reactivated lesions were consistent with the time course and CPE for standard

laboratory HSV cultures of low-quantity virus. The rate of culture positivity was in keeping with rates of virus recovery from other human and animal models.

Example 18 HSV PCR in Reactivated Animals

HSV pol gene segments were recovered by PCR from DNA prepared from early vesicular lesions (n = 4) but not from late, healed or crusting lesions. Samples that did not have evidence of HSV genetic material included skin samples from UV exposed uninfected mice (n = 2), uninfected non-UV exposed mice (n = 2), HSV infected non-UV exposed mice (n = 4), healing or crusted late reactivated lesions (n = 4), and healing or crusted late primary disease lesions (n = 2). Positive controls were DNA prepared from HSV-1 17 syn + and MacIntyre virus stock (Fig. 2). A 262 nt pol gene segment was amplified by PCR of DNA prepared from infected mouse skin samples and from viral DNA stock preparations. HSV DNA was recovered from early primary infection lesions and from early reactivated disease lesions. Templates were: Lane 1: molecular weight marker, Lane 2: HSV-1 MacIntyre strain, Lane 3: water blank, Lane 4: HSV-1 17 syn +, Lane 5: uninfected control mouse skin, Lane 6: skin from the non-exposed side of a UV reactivated mouse, Lane 7: skin from an infected, non-UV exposed mouse, Lane 8: late healing reactivated skin lesion, Lane 9: primary skin lesion, Lane 10: HSV-1 17 syn + DNA, Lanes 11 and 12: skin from early reactivated lesions in 2 separate mice, Lane 13: DNA from skin lesions in a fatal primary infection, and Lanes 14 and 15: molecular weight markers.

Example 19 Immunohistochemical studies of reactivated lesions

Frozen sections of skin lesions were evaluated for the presence of antigen. HSV-1 specific immunohistochemistry staining (IH) localized herpes antigen to epidermal lesions. There was positive lesion reactivity at an anti-HSV-1 antibody concentration of 1:12,000 in early reactivated lesions. At the same protein concentration, the irrelevant antibody and the control rabbit immunoglobulin produce diffuse light background staining of lesions, although the intensity of staining was considerably less than that seen with anti-HSV-1. We were unable to remove this spurious reactivity by absorption with powdered mouse skin. Within the epidermal lesions, both cytoplasm and nuclei of cells were immunopositive, with prominent nuclear staining. With maturation of lesions to ulceration and sloughing of surface epithelium, strong immunoreactivity disappeared.

Example 20 Effect of Sunscreens

The effect of sunscreens to inhibit HSV-1 reactivation on UVR exposure was evaluated. Following induction of a primary HSV-1 infection and healing as in Example 2, the animals were divided into three groups. One group received a formulated sunscreen with a UV-B protectant. A second group received a formulated sunscreen with a UV-A protectant. A control group received the sunscreen base, but with no UV protectant. The sunscreen was applied topically in the area of dermabrasion prior to UVR exposure of solar spectrum UV, as in Example 9. 5 of 6 control animals

receiving the sunscreen base with no UV protectant had HSV-1 reactivation. 2 of 5 animals receiving the UV-A suncreen protectant had HSV-1 reactivation. 0 of 5 animals receiving the UV-B sunscreen protectant had HSV-1 reactivation.

Example 21 Evaluation of Efficacy of Drug

Studies to determine the efficacy of a drug to be evaluated for inhibition of HSV reactivation infections or to limit the severity or duration of HSV reactivation infections are conducted. A primary HSV infection is induced as in Example 2 above. After allowing the superficial dermabrasion to heal and the HSV infection to resolve, animals are administered the drug to be tested. The area of superficial dermabrasion is exposed to UVR as in Example 9 above. The rate of HSV reactivation infection is determined, and optionally the severity or duration of HSV reactivation infection is determined.

Example 22 Dosing Studies

Animals are prepared as in Example 2 above. Dosing studies are conducted, in which the dose amount of drug administered, the total drug administered and the timing of administration relative to UVR exposure are varied. Subsequent to UVR exposure as in Example 9 above, the rate of HSV reactivation infection is determined, and optionally the severity or duration of HSV reactivation infection is determined.

Example 23 Route of Administration Studies

Animals are prepared as in Example 2 above. Route of administration studies are conducted, in which the route of administration, dose amount of drug administered, the total drug administered and the timing of administration relative to UVR exposure are varied. Routes of administration include injection, including i.v., i.m., s.c.,and i.p. injection, topical application, including cutaneous and mucousal application and oral administration. Subsequent to UVR exposure as in Example 9 above, the rate of HSV reactivation infection is determined, and optionally the severity or duration of HSV reactivation infection is determined.

Example 24 Two-Sided Infection Model

Animals are prepared as in Example 2 above, except that a primary infection is induced on both the left and right lateral abdominal walls. Following complete healing, the animals are used in studies of the efficacy of UV topical protectants. The UV topical protectant can be administered on either the left or right abdominal wall, with either a control preparation no containing a UV protectant, or no preparation, on the opposing abdominal wall. Reactivation is induced as in Example 9, preferably with solar spectrum UV. The rates of reactivation for each of the left and right abdominal walls, correlated to the UV topical protectant, are determined.

Example 25 Evaluation of Efficacy of Drug Administered Prior to or During Primary Infection

Studies are conducted to determine the efficacy of a drug to be evaluated for limiting the severity or duration of primary HSV infection, or a drug to be administered prior to or during primary HSV infection to inhibit subsequent HSV reactivation infections. A primary HSV infection is induced as in Example 2 above. At any time, prior to infection, prior to appearance of signs of HSV infection, or prior to resolution of the primary infection, a drug to be evaluated is administered. The efficacy of the drug for limiting the severity or duration of primary HSV infection can be determined by reference to controls not receiving the drug, and measurement of indicia of the severity and duration of the primary HSV infection. After allowing the superficial dermabrasion to heal and the primary HSV infection to resolve, the area of superficial dermabrasion is exposed to UVR as in Example 9 above. The rate of HSV reactivation infection is determined, and optionally the severity or duration of HSV reactivation infection is determined.

Example 26 Evaluation of Efficacy of Drug for Central Nervous System Protection

Studies are conducted to determine the efficacy of a drug, including but not limited to an anti-viral drugs, for providing central nervous system protection, including limitation or inhibition of spinal cord myelitis. A primary HSV infection is induced as in Example 2 above, except that higher quantities of innoculum are utilized, including quantities in excess of the LD 50 level, such that a primary HSV infection is induced with central nervous system involvement in at least a percentage of animals. A dose of one-half above LD 50 may be employed resulting in severe or fatal disease in approximately 75% of the animals and spinal cord disease in approximately 50% of the animals. At any time, prior to infection, prior to appearance of signs of HSV infection, or prior to resolution of the primary infection, a drug to be evaluated is administered. The efficacy of the drug for limiting or inhibiting spinal cord myelitis and other central nervous system involvement is evaluated.

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

Although the invention has been described in detail with particular reference to these preferred embodiments, other embodiments can achieve the same results. Variations and modifications of the present invention will be obvious to those skilled in the art and it is intended to cover in the appended claims all such modifications and equivalents. The entire disclosures of all references, applications, patents, and publications cited above are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. A method of determining the effectiveness of a composition to inhibit herpes simplex virus infection reactivation, comprising the steps of:

a) obtaining one or more animals;

b) creating an abrasion on the animal;

c) inoculating the animal with herpes simplex virus by application of a composition comprising herpes simplex virus to the abrasion, thereby resulting in a primary herpes simplex virus infection in the animal;

d) allowing the abrasion to heal and the primary herpes simplex virus infection to resolve;

e) administering a composition to be tested for inhibition of herpes simplex virus infection reactivation to the animal;

f) exposing the area of abrasion to radiation; and

g) determining whether the herpes simplex virus infection is reactivated.

2. A method of determining the effectiveness of a composition to inhibit herpes simplex virus infection, comprising the steps of:

a) obtaining one or more animals;

b) administering a composition to be tested for inhibition of herpes simplex virus infection to the animal;

c) creating an abrasion on the animal;

d) inoculating the animal with herpes simplex virus by application of a composition comprising herpes simplex virus to the abrasion; and

e) determining whether a herpes simplex virus infection resulted.

3. A method of determining the effectiveness of a composition to provide central nervous system protection, comprising the steps of:

a) obtaining one or more animals;

b) administering a composition to be tested to the animal;

c) creating an abrasion on the animal;

d) inoculating the animal with sufficient herpes simplex virus to induce central nervous system damage by application of a composition comprising herpes simplex virus to the abrasion; and

e) determining whether central nervous system damage resulted.

4. A method of determining an effective dose of a composition to inhibit herpes simplex virus reactivation, comprising the steps of:

a) obtaining two or more animals;

b) creating an abrasion on each animal;

c) inoculating each animal with herpes simplex virus by application of a composition comprising herpes simplex virus to the abrasion, thereby resulting in a primary herpes simplex virus infection in each animal;

d) allowing the abrasion of each animal to heal and the primary herpes simplex virus infection to resolve;

e) administering to each animal a selected dose of a composition to inhibit herpes simplex virus infection reactivation;

f) exposing the area of abrasion of each animal to radiation; and

g) determining the rate of reactiviation of the herpes simplex virus infection for each selected dose.

5. A method of determining the effectiveness of an ultraviolet protectant, comprising the steps of:

a) obtaining one or more animals;

b) creating one or more abrasions on the animal;

c) inoculating the animal with herpes simplex virus by application of a composition comprising herpes simplex virus to the abrasion, thereby resulting in a primary herpes simplex virus infection in the animal;

d) allowing the abrasion to heal and the primary herpes simplex virus infection to resolve;

e) administering an ultraviolet protectant to the animal;

f) exposing the area of abrasion to ultraviolet radiation; and

g) determining whether the herpes simplex virus infection is reactivated.

6. The methods of any of claims 1, 2, 3, 4 or 5 wherein the abrasion is a superficial dermabrasion.

7. The methods of any of claims 1 or 4 wherein the radiation is ultraviolet radiation, and is preferably a dose of two MED of ultraviolet-B radiation or solar spectrum ultraviolet radiation.

8. The method of any of claims 1, 2, 3, 4 or 5 wherein the herpes simplex virus is herpes simples virus-1 (HSV-1) or herpes simples virus-2 (HSV-2).

9. The method of any of claims 1, 2 or 4 wherein the herpes simplex virus is a strain isolated from a patient to be treated with a composition to inhibit herpes simplex virus infection reactivation.

10. The method of any of claims 1, 2, 4 or 5 wherein the quantity of HSV applied to the abrasion results in death of approximately 50% of animals administered said quantity of HSV, and is preferably at least one-half log less than the quantity of HSV which results in death of 50% of the animals.

11. The method of any of claims 1, 4 or 5, further comprising the step of determining the severity and duration of herpes simplex virus reactivation infection.

12. The method of claim 4, wherein at least two different selected doses are employed, with each animal administered one selected dose.

13. The method of claim 4, wherein the composition to inhibit herpes simplex virus infection reactivation comprises one or more active ingredients, and the quantity of active ingredient for each selected dose is varied.

14. The method of claim 2, further comprising the steps of:

f) allowing the abrasion to heal;

g) exposing the area of abrasion to ultraviolet radiation; and

h) determining whether a herpes simplex virus infection is reactivated.

15. The method of claim 5, wherein the ultraviolet protectant is a topical composition administered to at least one area of abrasion of the animal.

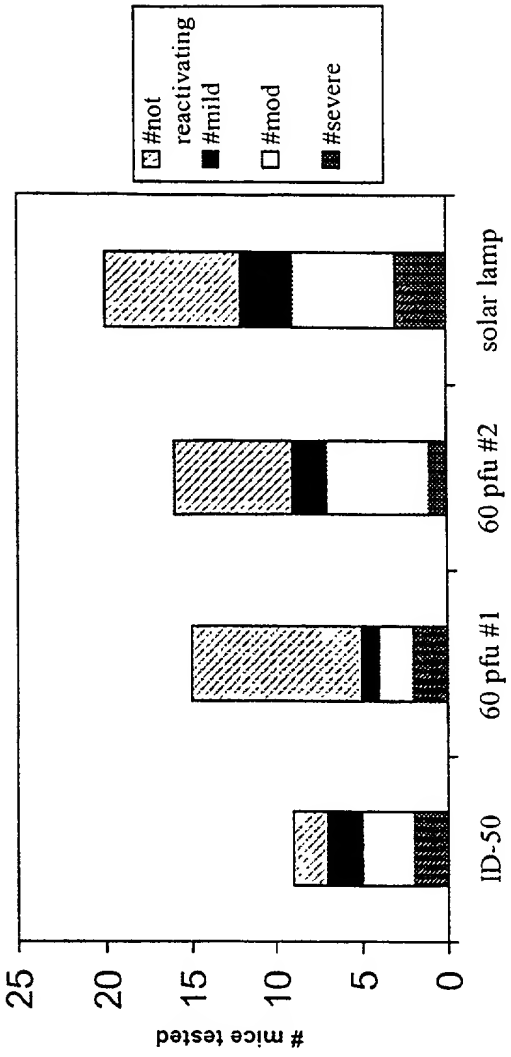
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Fig. 1

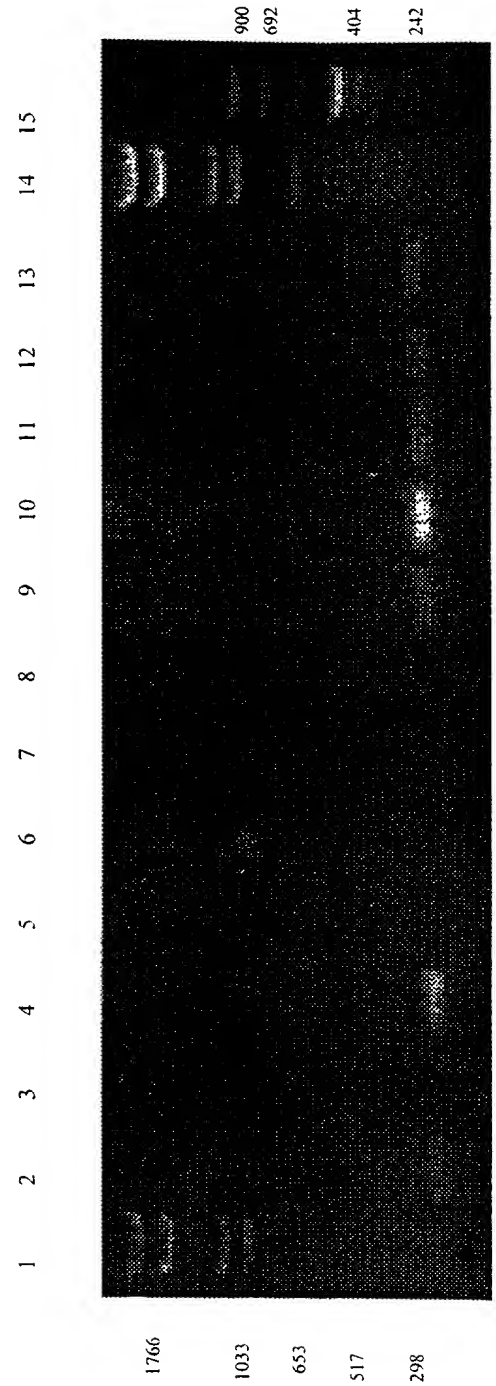
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Fig. 2

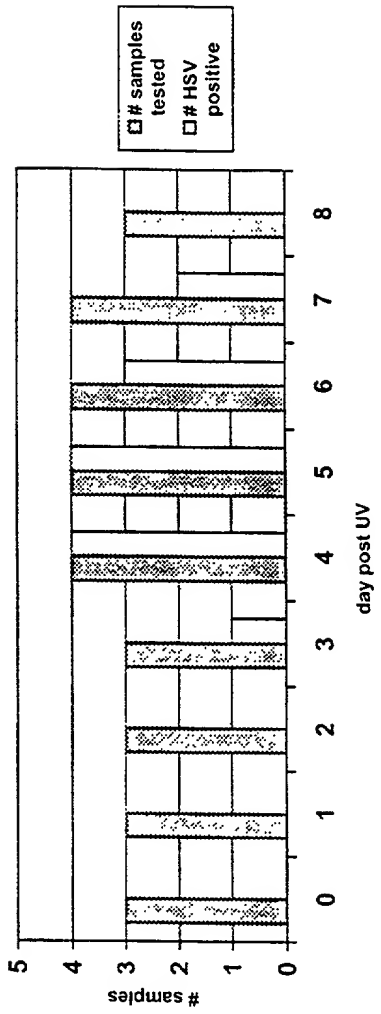

Fig. 3

Docket No.
MC-158.USA

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

HERPES SIMPLEX VIRUS REACTIVATION MODEL

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **August 30, 2001** as United States Application No. or PCT International Application Number 09/914,669

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)	
(Application Serial No.)	(Filing Date)	
(Application Serial No.)	(Filing Date)	

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

USSN 60/122,961	**03 March 1999**	**abandoned**
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/US00/05454	**03 March 2000**	**pending**
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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Full name of sixth inventor, if any
Sixth inventor's signature Date
Residence
Citizenship
Post Office Address

PATENT APPLICATION

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 via Label No. EL847395078US on August 30, 2001, addressed to **Box: PCT,** Commissioner for Patents, Washington, D.C. 20231.

Annette M. Turk, Legal Assistant — August 30, 2001 Date Signed

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	DIANE E. GOADE, DONNA F. KUSEWITT and ROBERT A. NOFCHISSEY	
Serial No.:	UNKNOWN	Attorney Docket No.: MC-158.USA
Filed:	HEREWITH (August 30, 2001)	Anticipated Group Art Unit: UNKNOWN
For:	HERPES SIMPLEX VIRUS REACTIVATION MODEL	

ASSOCIATE POWER OF ATTORNEY

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Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Stephen A. Slusher, a principal attorney in the above-identified application for Letters Patent, hereby appoints:

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Respectfully submitted,

Date: August 30, 2001

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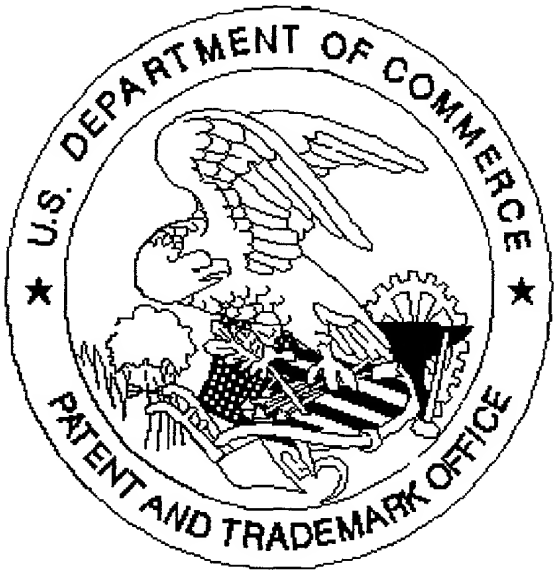

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☒ ***Scanned copy is best available.*** Figure 2 is dark.